## REMARKS

**Status of the claims.**

Claims 1-17 are pending, no claims being added or canceled with entry of this amendment. Claim 1 is amended herein. This amendment introduces no new matter. Support for this amendment is replete throughout the specification (*see, e.g.,* the abstract, Example X, page 6, lines 15-16, page 12, lines 1-4, *etc.*).

Claims 1-8 were rejected under 35 U.S.C. §112, first paragraph. Claims 1-17 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over various combinations of Nyirkos *et al.* (1990) *Biochem. J.* 268: 265, Johansen *et al.* (1991) *J. Bone Min. Res.,* 7(5): 501, Maurer *et al.* (1980) *Meth. Enzy.,* 70: 49, Campbell (1991) *Laboratory Techniques in Biochemistry and Molecular Biology*, 23: 1-113, and Serban *et al.* (U.S. Patent 4,782,014). Applicants respectfully traverse these rejections.

**Sequence Listing.**

The Examiner requested compliance with the sequence rules, 37 CF.R. §1.821-1.825. Accordingly, Applicants provide herewith a sequence listing and request entry of this amendment in adherence with 37 C.F.R. §§ 1.821 to 1.825. This amendment is accompanied by a floppy disk containing the sequences, SEQ ID NOs:1-4, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter. The amendments to the specification are made to substitute the formal sequence listing provided herewith for the sequence listing as filed.

**Information Disclosure Statement.**

The Examiner alleged that the Information Disclosure Statement filed on January 25, 2000 fails to comply with 37 C.F.R. §1.98(a)(3) because it does not include a concise explanation of the relevance of references A33-A36. The Examiner placed the references in the file, but has not considered these references.

Applicants respectfully submit that the Examiner's action is improper. 37 C.F.R. §1.98(a)(3) expressly requires:

> A concise explanation of the relevance, as it is presently understood by the individual designated in §1.56(c) most knowledgeable about

> the content of the information, of each patent, publication, or other information listed **that is not in the English language**. The concise explanation may be either separate from the specification or incorporated therein. [emphasis added]

References A33-A36 are clearly in the English language and contain descriptive material expressly identifying the listed sequence. Applicants are, thus, under no obligation to provide further explanation. Moreover, as the references were timely filed with the appropriate form 1449, the Examiner is obligated to consider these references and to make them of record. Accordingly Applicants request that references A33-A36 properly be considered and made of record.

**Formal Drawings.**

Applicants note with appreciation the Examiner's indication that formal drawings have been received and deemed acceptable.

**35 U.S.C. §112, first paragraph.**

Claims 1-8 were rejected under 35 U.S.C. §112, first paragraph, the specification while enabling for screening for the presence of a generic disease state associated with degradation of connective tissue containing YKL-40, allegedly does not reasonably provide enablement of the identification of specific diseases such as cirrhosis of the liver. Applicants respectfully traverse.

Applicants note that the same rejection was made in the parent application (USSN 08/581,527, now U.S. Patent 5,935,798, 06/24/97 Office action [paper 6], page 3, section 18). In that Office action, the Examiner suggested that amendment of the claims to recite "A method for screening for the presence of a disease state" would overcome this rejection.

In accordance with this previous suggestion Applicants have so amended the present claims to recite " A method of screening for a disease state associated with cirrhosis of the liver . . . ". The specification clearly teaches such a method. Example 10 provides detailed protocols by which blood from patient s with normal liver function and patients with liver disease are (*e.g.* alcoholic cirrhosis) patients are assayed for serum YKL-40 levels. Patients having liver disease (*e.g.* cirrhosis) showed a serum YKL-40 level 4 times greater than that observed in healthy patients. (*see, e.g.,* page 59, line 22, to page 61). **Applicants have clearly taught how to assay a patient for a disease state associated with cirrhosis of**

**the liver.** The application thus clearly meets the requirements of 35 U.S.C. §112, first paragraph, and the rejection on this ground should be withdrawn.

Moreover, the Examine is respectfully reminded that to be enabling under §112, first paragraph, a patent must contain a description that enables one skilled in the art to make and use the claimed invention. **That some experimentation is necessary does not constitute a lack of enablement**; the amount of experimentation, however, must not be unduly extensive.

Whether undue experimentation is required by one skilled in the art is typically determined by reference to eight factors considered relevant to the inquiry: (1) quantity of experimentation necessary; (2) amount of guidance presented; (3) presence of working examples; (4) nature of the invention; (5) state of the prior art; (6) relative skill of those in the art; (7) predictability of the art; and (8) breadth of the claims. *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988) *citing Ex parte Forman Inc.,* 230 USPQ 546 (BPAI 1986). Moreover, the Federal Circuit has held that routine screening (*e.g.,* screening antibodies for a particular binding specificity) is not undue experimentation. *Id.*

In the instant case, Applicants have provided guidance and examples detailing how to assay YKL-40 and how to distinguish normal from abnormal YKL-40 levels. This has been specifically exemplified for cirrhotic patients (Example X). The applicant thus clearly meets Wands/Forman factors 2 and 3. Having elevated YKL-40 levels, no experimentation (Wands factor 1) is necessary to distinguish rheumatoid arthritis, from cancer, from cirrhosis, *etc,* as this may be accomplished by routine differential diagnosis as explained in the specification (*see, e.g.* page 25, lines 24-27). Moreover, once elevated YKL-40 levels are determined a disease state associated with cirrhosis of the liver is detected. The state of the prior art is well developed with respect to immunoassays (Wands factor 5). The nature of the invention is a relatively straightforward assay (Wands factor 4) and the claims are relatively narrow being drawn specifically to YKL-40 assays (Wands factor 8). Moreover, in view of the extensive *in vivo* statistical information provided, the art is relatively predictable (Wands factor 7). Finally, it is noted that the level of skill in the art is very high (Ph.D./M.D.). An analysis of the *Wands* factors, particularly in view of the claims as amended herein, leads one to conclude that no undue experimentation is required to practice the claimed invention. Accordingly, the rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

**35 U.S.C. §103(a).**

Claims 9-11 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Nyirkos *et al.* (1990) *Biochem. J.* 268: 265 or Johansen *et al.* (1991) *J. Bone Min. Res.*, 7(5): 501, each in view of Maurer *et al.* (1980) *Meth. Enzy.*, 70: 49. Claims 12-17 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Nyirkos *et al.* or Johansen *et al., each in* view of Maurer *et al.* and further in view of Campbell (1991) *Laboratory Techniques in Biochemistry and Molecular Biology*, 23: 1-113. Applicants note however, that the Examiner failed to discuss Campbell and instead discussed Serban *et al.* (U.S. Patent 4,782,014). Accordingly, Applicants assume that the Examiner misstated his rejection and understand Serban to be cited instead of Campbell.

In particular, the Examiner allegedly that teach the quantitation and detection of a 39 kD protein and that Applicant's own publication (Johansen *et al.* (1991) J. Bone Min. Res., 7(5): 501) equates YKL-40 with the protein of Nyirkos. Johansen *et al.* is cited as allegedly teaching the quantitation of YKL-40 from human osteosarcoma cells in SDS gels. According to the Examiner, Nyirkos *et al.* and Johansen *et al.* differ from the instant invention in that they do not specifically teach the use of polyclonal or monoclonal antibodies as a means to measure YKL-40. The Examiner cites Maurer *et al.* as allegedly teaching that monoclonal antibodies can be produced against virtually any macromolecule. Serban *et al.* is cited as allegedly teaching test kits for a number of acute and chronic inflammatory diseases such as rheumatic conditions. Applicants respectfully traverse.

The Examiner is respectfully reminded that a *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. When a rejection depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Geiger*, 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally provide a reasonable expectation of success. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

In the instant case, Applicants explain below that Nyirkos *et al.* and Johansen *et al.* **fail** to describe a relationship between YKL-40 level and cirrhosis of the liver. In addition, these references only describe the behavior of cells **in culture** and this is not predictive of the behavior of cells *in vivo*. Moreover, numerous other proteins are secreted by cells described in these references and yet are unsuited for use as diagnostic markers. Finally,

Applicants note that comparisons with normal controls are necessary to establish that a marker can reliably distinguish between normal and pathological conditions. For these reasons the combination of the cited references fails to provide all the elements of the claimed invention, fail to provide motivation to produce the presently claimed invention, and fail to provide a reasonable expectation of success.

**A) The cited art fails to identify a relationship between YKL-40 and cirrhosis of the liver.**

Nyirkos *et al.* and Johansen *et al.* fail to disclose a relationship between YKL-40 and cirrhosis of the liver. Nyirkos *et al.* teaches that a major secreted protein of human articular chondrocytes in monolayer or explant culture or of synovial fibroblasts is HC gp-39 (also known as YKL-40). The reference further states that "under normal physiologic conditions, the liver **could be** a major source of this protein." (see page 25808, column 2). The liver, however is not definitively associated with YKL-40 and Nyirkos *et al.* offers no teaching or suggestion that YKL-40 may be associated with a cirrhotic state. To the contrary, **Nyirkos *et al.* completely fails to mention cirrhosis.**

Similarly, Johansen *et al.* teach that YKL-40 is produced by osteosarcoma cells (MG-63 cells) in culture. Johansen *et al.* only associates YKL-40 with osteoblasts. This reference fails to mention liver or cirrhosis of the liver. The combination of Nyirkos *et al.* and Johansen *et al.* thus fails to establish any relationship whatsoever between YKL-40 production (secretion) and cirrhosis of the liver.

This defect is not remedied by Maurer *et al.*, Campbell, or Serban *et al.* Maurer *et al.* Maurer *et al.* generally describes the preparation of monoclonal antibodies and fails to even mention YKL-40 or cirrhosis of the liver. Similarly, Campbell is a generally a molecular biology reference book and also fails to even mention YKL-40 or cirrhosis of the liver. Serban *et al.* simply describers immunological analysis for serum amyloid A protein (SAA) and serum amyloid P-component (SAP). Again there is no teaching or suggestion of a relationship between YKL-40 and cirrhosis of the liver. The cited art, individually or in combination, simply fails tech or suggest a relationship between YKL-40 level and cirrhosis of the liver and thus fails to teach or suggest all the limitations of the presently claimed invention. The Examiner has therefore failed to make his *prima facie* case and the rejection of claims 1-17 under 35 U.S.C. §103(a) should be withdrawn.

**B) The cited art fails to teach a viable assay because in vitro conditions are not predictive of *in vivo* cellular behavior.**

**1) It is generally known that protein secretion is altered by environmental conditions.**

By the priority date of the present application, it was well known that synovial cells (*e.g.*, synoviocytes) secrete a wide variety of compounds including, but not limited to, hyaluronic acid and proteins such as stromelysin, collagenase, inhibitors of metalloproteinase(s). It was also known that the biosynthesis of these and other proteins is controlled by a wide variety of exogenous and endogenous factors. As explained by Hakala *et al.* (1993) *J. Biol. Chem.*, 268(34): 25803-25810 (attached as Exhibit A), such factors include:

1) Differentiation status of the cells

2) The presence of growth factors and cytokines;

3) Appropriate stimulation;

4) The presence of secondary mediators (*e.g.*, TGF-β, insulin-like growth factors, IL-8, Platelet factor family members, *etc.*);

5) The extracellular environment of the cells (*e.g.* to compensate for changes in structure and functionality of the matrix. (*see, e.g.* Hakala *et al.* Enclosed as Exhibit A).

In view of the foregoing, and equipped with the general knowledge that the *in vitro* culture milieu differs significantly from the *in vivo* environment of the cells, one of ordinary skill would not expect that the protein secretion profiles of synovial cells *in vitro* would necessarily reflect those observed *in vivo*. To the contrary, in view of the above-listed factors, one of skill would generally expect that the secretion profiles of cells in culture would likely differ from those observed *in vivo*.

**2) YKL-40 secretion profiles in particular are altered by cell culture.**

Moreover, YKL-40 levels in particular, are altered by cell culture. For example, Hakala *et al.* (Exhibit A), published after the filing date of this application and therefore not prior art, teaches that gp-39 is not detectable in normal newborn or adult human

articular cartilage obtained at surgery (*see,* Hakala *et al.* abstract) or within culture media after 24 hours of culture (Hakala *et al.* at 25805, column 2, top). However, after three days of culture, YKL-40 was detected in culture media (*Id.*). Thus, Hakala *et al.* teaches that the simple act of culturing cells can significantly alter their protein secretion profile. Hakala *et al.* thus supports the view held in the prior art that culture conditions can and do alter the secretion properties of cells and the activity of cells in culture is not predictive of their activity *in vitro.*

Johansen *et al.* even expressly states:

> In addition, the conditions osteoblasts encounter in cell culture are far from those they typically encounter in vivo, and therefore **the relationship between the present results and the pattern of protein expression in situ remans to be established.** [emphasis added] (Johansen *et al.*, page 508 column 2 - page 510 column 1).

Thus, even assuming *arguendo*, that the cited references taught a relationship between YKL-40 and liver cirrhosis (which they do not), the *in vitro* activity of synovial cells reported by Nyirkos *et al.* and the *in vitro* activity of osteosarcoma cells reported by Johansen *et al.*, is not sufficient provide a reasonable expectation that YKL-40 can successfully be used as a diagnostic marker for any *in vivo* condition. For this reason, as well, the Examiner has failed to make his *prima facie* case and the rejection of claims 1-17 under 35 U.S.C. §103(a) should be withdrawn.

**C) Without appropriate normal *in vivo* controls, culture data does not indicate that a marker can distinguish between a pathological and a healthy state.**

The cited art provides no *in vivo* measure of YKL-40 and provides no comparison of YKL-40 levels from normal tissues with YKL-40 levels from pathological tissues. Indeed, in the cited references, normal tissue levels of YKL-40 do not appear to have been measured. Without appropriate normal and pathological *in vivo* controls, cell culture data does not indicate that a marker can distinguish between a pathological and a healthy state.

The cells of the synovial membrane secrete a large number of proteins including, but not limited to, collagenase and other matrix components (like hyluronan), proteinases (like metalloproteinases, the cathepsins, elastase, urokinase-type plasminogen

activators, proteinase inhibitors, cytokines, and growth factors. Many of these proteins are not found in synovial fluid and thus unsuitable for diagnostic markers. For example, Applicants' own immunohistochemical analysis of the synovial membrane from rheumatoid arthritis patients demonstrate that most of the YKL-40 positive cells also express the monocyte/macrophage antigen CD68. However, to date, CD68 has not been detectable in synovial fluid or sera and has therefore not proven to be a useful diagnostic marker.

**D) Lacking the proper statistical analysis, *in vitro* data fails to establish that a marker can distinguish between healthy and pathological states.**

A comparison of YKL-40 secretion levels in pathological conditions to YKL-40 secretion levels in a healthy organism is required to establish that the marker can distinguish the two different states. Such data is provided in the present application, but is not found in the cited art. The Examiner has failed to identify any teaching the cited art establishing that YKL-40 levels are elevated in a disease state as compared to a healthy state. The Examiner, is respectfully reminded that not only do culture conditions alter the expression and secretion of proteins, but the state of health of the cells also alters the protein secretion profile.

The cited art fails to provide any comparison whatsoever with normal YKL-40 levels. There is no measure of YKL-40 variability in healthy or pathological tissue. Lacking such a comparison and consequently any statistical analysis, the cited art simply fails to establish that YKL-40 levels can be used to distinguish a healthy from a diseased state.

In view of the foregoing, the cited art fails to offer any teaching or suggestion of the claimed invention or any reasonable expectation of success. The Examiner has failed to make his *prima facie* case and the rejection of claims 1-17 under 35 U.S.C. §103(a) should be withdrawn.

In view of this discussion, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (415) 217-6021.

Respectfully submitted,

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Encl: 1) Petition for 3 month extension of time.
2) Sequence listing paper copy and disk.
3) Exhibit A: Hakala et al. (1993) J. Biol. Chem., 268(34): 25803-25810
4) Change in correspondence address.

## APPENDIX I

## CLAIMS PENDING IN 08/746,207WITH ENTRY OF THIS AMENDMENT

1. (Once amended) A method of [identifying the presence of]screening for a disease state associated with cirrhosis of the liver in a mammal, said method comprising measuring the level of YKL-40 in a biological sample of the mammal and comparing the level to that of a normal, healthy mammal, wherein a statistically significant difference indicates the presence of said [cirrhosis]disease state.

2. The method of claim 1, wherein the amount of YKL-40 in said sample is measured using an immunoassay.

3. The method of claim 2, wherein the immunoassay is a competitive immunoassay.

4. The method of claim 3, wherein the immunoassay utilizes a detectable label selected from the group consisting of radioisotopes, enzymes, fluorescent molecules, chemiluminescent molecules, bioluminescent molecules, and colloidal metals to measure YKL-40.

5. The method of claim 1, wherein said mammal is a human.

6. The method of claim 2, wherein the immunoassay uses a polyclonal antibody to measure YKL-40.

7. The method of claim 2, wherein the immunoassay uses a monoclonal antibody to measure YKL-40.

8. The method of claim 1, wherein said sample is selected from the group consisting of blood, plasma, and serum.

9. Antibodies that specifically bind to a YKL-40.

10. The antibodies of claim 9, wherein said antibodies are polyclonal antibodies produced by immunization of a non-human mammal.

11. The antibodies of claim 9, wherein said antibodies are monoclonal antibodies produced by hybridomas formed from cells taken from a non-human mammal.

12. A kit for use in the detection of a disease state in a mammal associated with degradation of connective tissue, said kit comprising a YKL-40 antibody.

13. The kit of claim 12, wherein said disease state is selected from the group consisting of an inflammatory or degenerative joint disease, cirrhosis of the liver, and metastatic cancer.

14. The kit of claim 12, wherein said YKL-40 antibody is a polyclonal antibody.

15. The kit of claim 12, wherein said YKL-40 antibody is a monoclonal antibody.

16. The kit of claim 12, further comprising immunoassay reagents.

17. The kit of claim 12, further comprising a detectable label.